

a³ broad, preferably about 1: 1, although other proportions may also be utilized depending on the type of lipid agent and the DNA utilized. This proportion is not crucial.--.

At page 20, lines 15-22, please delete the entire paragraph and insert therefor the following:

a⁴ --The GFP DNA-transferrin-polylysine viral complexes, prepared as described in Example 4 above, were delivered into the seminiferous tubules of three (3)-week-old B6D2F1 male mice. The DNA delivery by transferrin receptor-mediated endocytosis is described by Schmidt et al. and Wagner et al. (Schmidt et al., Cell 4: 41-51 (1986); Wagner, E., et al. PNAS (1990), (USA) 81: 3410-3414 (1990)). In addition, this delivery system relies on the capacity of adenoviruses to disrupt cell vesicles, such as endosomes and release the contents entrapped therein. The transfection efficiency of this system is almost 2,000 fold higher than lipofection.--.

IN THE CLAIMS:

Please cancel Claims 1-134, without prejudice, as originally filed with parent application 09/191,920, and add the following new Claims 135-155 as being directed to the subject matter of designated claim Group III, which is herein elected.

--135.(New) An in vitro method of incorporating at least one polynucleotide encoding a desired trait into a male germ cell, comprising

a⁵ obtaining a male germ cell from a non-human vertebrate, said germ cell being selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, spermatids, and spermatozoa;

transfecting the germ cell in vitro with at least one polynucleotide encoding a gene product in operable linkage with a promoter, in the presence of a gene delivery mixture comprising at least one transfecting agent, and optionally a polynucleotide encoding a genetic selection marker; and

allowing the polynucleotide encoding a gene product to be taken up by, and released into the germ cell.

136.(New) The method of Claim 135, further comprising allowing the incorporation of the released polynucleotide into the genome of the germ cell.

137.(New) The method of Claim 135, wherein the male germ cell is a spermatogonial cell or other undifferentiated male germ cell.

138.(New) The method of Claim 135, wherein the transfection is conducted under conditions of temperature of about 25°C to about 38°C.

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139.(New) The method of Claim 135, wherein the transfecting agent is selected from the group consisting of liposomes, viral vectors, and other uptake enhancing DNA segments, or comprises a mixture of any members of said group.

140.(New) The method of Claim 139, wherein the viral vector is selected from the group consisting of retroviral vectors, adenoviral vectors, transferrin-polylysine enhanced adenoviral vectors, Moloney murine leukemia virus-derived vectors, mumps vectors, and virus-derived DNAs that enhance polynucleotide uptake by and release into the cytoplasm of germ cells, or an operative fragment of- or mixture of any members of said group.

141.(New) The method of Claim 140, wherein the retroviral vector is selected from the group consisting of lentiviral vectors.

142.(New) The method of Claim 135, wherein the transfecting agent comprises an adenovirus vector having endosomal lytic activity, and the polynucleotide encoding a gene product is operatively linked to the vector.

143.(New) The method of Claim 135, wherein the polynucleotide encoding a gene product is in the form of a complex with a viral vector.

144.(New) The method of Claim 135, wherein the transfecting agent comprises a lipid transfecting agent.

145.(New) The method of Claim 135, wherein the transfecting agent further comprises an agent selected from the group consisting of a c-kit ligand and at least one genetic selection marker; and

the method further comprises isolating or selecting a male germ cell carrying at least one polynucleotide encoding a gene product and at least one polynucleotide encoding a genetic selection marker, from a donor male vertebrate with the aid of the genetic selection marker.

146.(New) The method of Claim 145, wherein the genetic selection marker comprises a gene expressing a detectable product, driven by a spermatogonia-specific promoter selected from the group consisting of c-kit promoter, b-Myb promoter, c-raf-1 promoter, ATM (ataxia-telangiectasia) promoter, RBM (ribosome binding motif) promoter, DAZ (deleted in azoospermia) promoter, XRCC-1 promoter, HSP 90 (heat shock gene) promoter, and FRMI (from fragile X site) promoter.

147.(New) The method of Claim 135, wherein the non-human vertebrate is a mammal.

148.(New) The method of Claim 147, wherein the mammal is selected from the group consisting of non-human primates and farm and marine mammals.

149.(New) The method of Claim 135, wherein the polynucleotide encoding a gene product is derived from the same non-human vertebrate species as the germ cell.

150.(New) The method of Claim 135, wherein the non-human vertebrate is selected from the group consisting of wild and domesticated vertebrates.

151.(New) The method of Claim 135, wherein the polynucleotide encoding a gene product is derived from a non-human mammal selected from the group consisting of human and non-human primates, canines, felines, swines, farm mammals, pachyderms, marine mammals, equines, murine, ovine and bovine, or from a bird selected from the group consisting of ducks, geese, turkeys and chickens.